Scientific Research Publishing

Food and Nutrition Sciences, 2018, 9, 1285-1298
http://www.scirp.org/journal/fns
ISSN Online: 2157-9458
ISSN Print: 2157-944X

Efficacy of *Moringa oleifera* Leaf Supplementation for Enhanced Growth Performance, Haematology and Serum Biochemistry of Rabbits

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How to cite this paper: Aljohani, N.E. and Abduljawad, S.H. (2018) Efficacy of *Moringa oleifera* Leaf Supplementation for Enhanced Growth Performance, Haematology and Serum Biochemistry of Rabbits. *Food and Nutrition Sciences*, **9**, 1285-1298.
https://doi.org/10.4236/fns.2018.911092

Received: October 17, 2018
Accepted: November 19, 2018
Published: November 22, 2018

Abstract

The present work aimed to define the antioxidant effect of addition graded levels (0 mg (T0), 500 mg (T1), 1000 mg (T2) /Kg diet) of dried *M. oleifera* leaves (DMOL) during the experimental period for 56 days duration on the productive, hematology and some serum biochemical parameters of fifty-one growing rabbits. The results indicated that the DMOL contents of chlorophyll a, b, vitamin c, vitamin E, total flavonoids, total polyphenols, condensed tannins and phytic acid were (1.09 mg/g DW), (0.34 mg/g DW), (0.95 mg/g), (0.75 mg/g), (5.06 mg/g), (2.32%), (1.72%) and (0.98%), respectively. The result of the final live weight, average daily weight gain (ADWG) and average daily dry matter intake increased significantly with increasing levels of DMOL in diets. Also, the improvement in feed conversion ratio (FCR) was significantly high among the two levels of (DMOL). The serum glucose and urea nitrogen levels significantly decreased as DMOL levels increased. Moreover, there were a significant increase ($P < 0.05$) in Aspartate Aminotransferase (AST), alkaline phosphatase (ALT) and Alkaline phosphatase (ALP) with control group compared with groups 2 and 3. Also, total cholesterol values decreased significantly ($P < 0.05$) by the addition of DMOL in group 3 (19.03%) and group 2 (10.27%) compared to control group. White blood cell count, red blood cells and PLT values of control group were significantly lower ($P < 0.05$) than that of groups 2 and 3. Values of Total Volatile Fatty Acids (TVFA) and caecal pH were significantly ($p > 0.05$) different among treatments. On the contrary, significantly ($P < 0.05$) lowest values of NH3-N were observed in rabbits fed diet 3 (23.84 mg/dl), diet 2 (26.65 mg/dl) and lastly control diet (28.51 mg/dl). The total count of bacteria of ceacal content of rabbits in group T2 was significantly ($P < 0.05$) lower than those in control

group T0 but similar ($P > 0.05$) to the values of group T1. There was a significant ($P < 0.05$) effect of DMOL supplementation on the percent of carcass, liver and total edible parts of rabbits across treatments, whereas, treatments T2 and T1 recorded better results than T0. Under the condition of the present study, the results suggest that DMOL supplementation up to 1000 g/Kg diet might improve performance, bacterial community, antioxidant, biochemical parameters and blood constituents of rabbits.

Keywords

Moringa oleifera, Growth Performance, Biochemical Activity, Carcass, Cacum Activity

1. Introduction

Free radicals play a major role in the pathogenesis of many human diseases such as cardiovascular and cancer diseases [1]. Interestingly, natural antioxidants present in food of plant origin such as *Moringa oleifera* are important tools in obtaining and preserving good health [2].

Moringa oleifera leaves contain a number of important vitamins A, B complex (B1, B3, B6 and B7), C, D, E, K, specific plant pigments with a good profile of amino acids and minerals (Ca, P, and Fe) [3] [4] [5] and negligible contents of secondary metabolites [6]. In addition, *M. oleifera* families are known to contain rich antioxidant compounds [7], it was also used an antimicrobial agent [8] to promote the immune system against various infections [9].

The biological activities of these plants are due to the presence of phytochemicals (flavonoids and other phenolics) in their leaves [10]. The protection against these radicals by natural antioxidants may be done by enhancing the activities of anti-oxidant enzymes, reducing the intensity of lipid peroxidation and inhibiting generation of free radicals [11].

The aim of the present study was to find out the antioxidant effect of addition graded levels of dried *M. oleifera* leaves (DMOL) on the productive, haematology and plasma biochemical parameters of growing rabbits.

2. Material and Methods

The experimental work was carried out at, Animal house of King Khalid hospital, Kingdom of Saudi Arabia (KSA). Packaged Moringa leaf powder (250 g) was purchased from the Moringa plantation unit of the Scientific Association of Moringa, National Research Center, Egypt.

2.1. Management and Feeding of the Experimental Rabbits

Fifty-one weaner New Zealand white rabbits were randomly allotted to three experimental treatments with seventeen animals each in a Completely Rando-

mized Design. Each rabbit received an assigned diet for eight weeks (56 days). The rabbits were given ad libitum drinking clean water and the control diet (T0) while treatments T1 and T2 contained 500 mg and 1000 mg of DMOL per one kg of feed for treatments respectively (Table 1). Whereas, the experimental diets T1 and T2 were prepared by adding fine powder of *Moringa oleifera* leaf leaves to the soft ingredients which included Limestone, Di-Ca-phosphate, DL-Methionine, NaCl, Vit.-Min. premix of the experimental diets and was then well mixed to get perfectly homogenous experimental diets. The experimental diet (Control) contained Soybean meal (44% CP) (20.9%), Barley (32%), Wheat bran (9.2%), berseem hay (31%), Molasses (3%), Limestone (0.7%), Di-Ca-phosphate (2.2%), DL-Methionine (0.4%), NaCl (0.3%), Vit.-Min. premix (0.3%). The composition of the control diet and DMOL analyzed nutrient content are shown in Table 1.

2.2. Growth and Carcass Characteristics

The all rabbits were kept under similar conditions of management and weighed at the beginning of the trial and every 14 days which used to calculate an average daily weight gain. The average daily feed intake and feed conversion (g feed/g gain) rate were recorded. The amount of feed offered as well as any feed left over were daily weighed and recorded for each animal during the experimental period to calculate average daily feed intake (ADFI). At the end of the experiment (56 day), five rabbits from each treatment were randomly selected, fasted for 18 hours and slaughtered by cutting the jugular vein. Live body weights were recorded prior to slaughter. After evisceration, the evaluation focused on organ weights such as the carcass and giblets (liver, Kidney and heart) and were separately weighed.

2.3. Analytical Procedure

Feed samples and the fine powder of *Moringa oleifera* leaf were taken every week for proximate analyses [12] which are presented in Table 1.

The DMOL content of chlorophylls a and b were determined using spectrometric method described by Dere *et al.* [13]. Ascorbic acid was assayed by the method described by Khan *et al.* [14] by using HPLC method. The condensed tannins were determined according to Makkar [15], total phenolic content was determined using Mc. Donald, *et al.*, [16] whereas, flavonoids estimated by method of Kumaran and Karunakaran [17]. The free radical scavenging activity of DMOL on DPPH radical was estimated using the method described by Liyana-Pathiranan *et al.* [18] at 515 nm. Bioactive compounds, antinutritional components and antioxidant potential of DMOL are presented in Table 2.

2.4. Blood Sample

At the end of the feeding trial, blood samples (4 ml) were collected from the ear veins into individually marked vials from each animal on the last day of the

Table 1. Proximate compositions of the experimental diet and *Moringa oleifera* leaves.

Constituents (%)	Control group	*Moringa oleifera* leaves
DM	92.88	92.05
OM	90.88	90.25
CP	17.56	22.54
CF	13.46	17.09
EE	2.3	4.65
NFE	57.56	45.97
Ash	9.12	9.75

Table 2. Bioactive compounds, antinutritional components and antioxidant potential of dried *Moringa Oleifera* leaves.

Items	Dried *M. Oleifera* leaves
Bioactive compounds	
Chlorophyll A (mg/g DW)	1.09
Chlorophyll B (mg/g DW)	0.34
Vitamin c (mg/g)	0.95
Vitamin E (Tocopherol) (mg/g)	0.75
Total polyphenols (%)	2.32
Total flavonoids, (mg/g)	5.06
Antinutritional component	
Condensed tannins (%)	1.72
Phytic acid (%)	0.98
Antioxidant potential	
DPPH scavenging activities, %	78.05

study. The samples were separated into two lots and used for biochemical and haematological studies.

Firstly, an initial 2.0 ml blood samples were collected into test tubes without anticoagulant were centrifuged at 3000 rpm for 10 minutes in a microcentrifuge to obtain serum which kept at –20˚C until analysis which used to determine the biochemical components using a spectrophotometer at a wavelength of 500 nm. Serum contents of cholesterol and liver enzymatic activity (aspartate amino-transferase, (AST), alanine aminotransferase, (ALT) and alkaline phosphatase, (ALP) were measured calorimetrically using commercial kits (purchased from Bio-diagnostic, Cairo, Egypt) according to the manufacturers' instructions. Serum glucose, urea nitrogen and creatinine were determined according to Fawcett and Soctt [19]. Total protein was determined according to Orsonneau *et al.* [20]. Albumin was determined according to the method of Doumas *et al.* [21]. Serum globulin concentration was calculated by the difference between total protein

and albumin. Secondly, for haematological analysis, another 2.0 ml of the blood samples was collected into test tubes with anticoagulant were centrifuged at 3000 rpm for 10 minutes in a microcentrifuge to obtain serum and kept at –20˚C until analysis. Serum contents of blood haemoglobin concentration (HGB), white blood cell count (WBC), thrombocyte count (PLT) using commercial kits were determined by standard procedures described by Davice and Lewis [22].

2.5. Caecum Activity and Microbial Activity Estimation

Immediately after the slaughter, the cecum and colon contents were individually removed from five slaughtered rabbits from each group, the cecum was weighted. The fresh cecum pH was determined instantly after slaughtering using digital pH meter (Orion Research Digital pH meter, model 201) and then the caecal content divided into two samples, one of them was taken to estimate the total anaerobic bacteria count determined by Standard method according to Kim and Goepfert [23] using nutrient agar medium [24], another sample was filtered through four folds of gauze for determination of total volatile fatty acids (VFA) and ammonia nitrogen by steam distillation according to Warner [25]. Total bacterial counts were determined under strict anaerobic conditions according to the method described by Houghtby *et al.* [26].

2.6. Statistical Analysis

Data were statistically analyzed using One-Way Layout with Means Comparisons Procedure SAS [27]. Differences among means were tested by Duncan's Multiple Range Test [28].

3. Results and Discussion

The bioactive compounds, antinutritional components and antioxidant potential of DMOL are presented in **Table 2**. The DMOL contents values were of chlorophyll a (1.09 mg/g DW), b (0.34 mg/g DW), vitamin c (0.95 mg/g), vitamin E (0.75 mg/g), total flavonoids (5.06 mg/g) and total polyphenols (2.32%). Quantitative secondary metabolites estimated in the water leaf extract showed condensed tannins of 1.72% and phytic acid of 0.98%. The percentage DPPH scavenging activities of *Moringa oleifera* leaves extract are 78.05, and this increase may be attributed to its hydrogen donating ability of the DMOL.

As presented in **Table 3**, no statistically significant difference was found for average initial live weight between experimental diets. The result of the final live weight, average, daily weight gain (ADWG) and average daily dry matter intake increased significantly with increasing levels of dried *M. oleifera* leaves in diets and the values of rabbit's weight gains were close to those obtained (21.5 and 22.6 g/d) by Kpodékon *et al.* [29] and Kpodékon *et al.* [30]. These findings are in line with the published reports before of rabbits [31] [32] [33] who found that *M. oleifera* leaves supplementation could play a good impact on growth performance of rabbits with low levels but adverse effects were observed with high levels of *M. oleifera* leaves supplementation.

Table 3. Effect of dried *Moringa oleifera* leaves on growth performance and feed conversion ratio of rabbits.

Parameters	T0	T1	T2	±SE
Number of animals	17	17	17	
Av. Initial live weight (g)	671.81a	671.08a	669.10a	3.10
Final live weight (g)	2045.93c	2179.68b	2333.42a	9.63
Total weight gain (g)	1374.12c	1508.6b	1664.32a	6.41
Av. Daily weight gain (g)	24.54c	26.94b	29.72a	0.08
Av. daily dry matter intake (g)	70.13c	74.91b	80.54a	0.16
Feed conversion ratio	2.86a	2.78b	2.71c	0.02

a, b and c: Means in the same row having different superscripts differ significantly ($P < 0.05$).

The improvement of productive performance may be due to the increase in feed intake, the fact that *M. oleifera* is rich in amino acids, vitamins especially vitamin A [34] and minerals particularly iron [35], the biological function of *M. oleifera* that have been natural substances which can promote health and alleviate illness. Also, this improvement may be due to *M. oleifera* was used as antimicrobial agent [36] which might enhance utilization of nutrients. Also, the improvement in feed conversion ratio (FCR) was significantly high among the two levels of dried *M. oleifera* leaves while the best FCR was recorded in diet 3 (5.24%) and diet 2 (2.81%) compared to control diet that means better returns on investment. This might be due to the presence of bioactive compounds in *Moringa oleifera* leaves as reported by Lannaon [37] and antibacterial and antioxidant activities of *Moringa oleifera* leaves [38]. This result is in harmony with those of Alabi *et al.* [39] who found that addition of 90 mL and 120 mL of aqueous *Moringa oleifera* leaf extracts per liter in broilers diet produced better feed conversion than control diet.

3.1. Serum Biochemical Parameters

As shown in **Table 4**, total protein, albumin and globuline levels determined in this study were significantly reduced ($P < 0.05$) in rabbits of group 3 and group 2 compared to control rabbits and these values are generally influenced by the quality and quantity of protein intake [40]. These observations of albumin and globulin fall within the range of reference values reported for healthy rabbits in previous studies [41] [42] which is an indication of normal systemic protein utilization of the liver [43] for albumin and high immunity in the experimental animals for globulin [44]. These results agree with Asiedu-Gyekye *et al.*, [45] who showed that supplementation of raw powder of DMOL (40 mg /kg) did not show any pathological alteration in rats.

As can be seen from the **Table 5**, the serum glucose and urea nitrogen levels significantly decreased as DMOL levels increased. No statistically significant difference for serum creatinine was determined in this study between rabbits of T1

Table 4. Effect of dried *Moringa oleifera* leaves on serum biochemical parameters of growing New Zealand rabbits.

Items	Treatments			± SE
	Control	T1	T2	
Total Protein, (g/dl)	8.15a	7.57b	7.03c	0.04
Albumin, (g/dl)	4.04a	3.76b	3.41c	0.03
Globuline, (g/dl)	4.11a	3.81b	3.62c	0.03
Glucose, (mg/dl)	95.46a	88.29b	85.87c	0.15
Urea nitrogen, (mg/dl)	39.72a	35.81b	32.19c	0.10
Creatinine, (mg/dl)	0.68a	0.61b	0.57b	0.01
Aspartate aminotransferase, AST (U/L)	98.11	99.54	102.03	1.49
ALT (U/L) Alanine aminotransferase	62.83	63.09	63.91	1.08
Alkaline phosphatase, ALP (U/L)	125.84	124.95	124.57	0.47
Total cholesterol (mg/100ml)	65.65a	58.91b	53.16c	0.16
Triglyceride, TG	0.38a	0.31b	0.28b	0.02

Table 5. Effect of dried *Moringa oleifera* leaf on some haematological parameters of growing New Zealand rabbit.

Items	Treatments			SE
	T0	T1	T2	
white blood cell count, WBC (×109/L)	7.84c	8.91b	10.86a	0.13
Red blood cells, RBCs (×1012/L)	5.21c	5.91b	6.48a	0.03
PLT (×109/L)	645a	792b	845c	3.99
whole blood haemoglobin concentration, Hb (g/dl)	11.65c	12.36b	12.81[a]	0.11

a, b and c: Means in the same row having different superscripts differ significantly ($P < 0.05$).

and T2 but was significantly higher ($P < 0.05$) for control group. Moreover, these results agree with the findings of Jaiswal *et al.* [9] who indicated that blood glucose level decreased after supplementation of *Moringa oleifera* aqueous leaf extract to rats that might be due to *Moringa oleifera* leaf has some effects of increasing the tissue utilization of glucose [46] or by inhibiting gluconeogenesis [47]. The low level of blood urea in the test animals are associated with high protein quality [48] which are indication that the amino acids of *M. oleifera* are balanced [49]. Also, Omobowale *et al.*, [50] reported that the low blood urea observed in Wister rats received 400 mg/kg body of methanol extract of *Moringa Oleifera* for 5 weeks.

Moreover, there were a significant increase ($P < 0.05$) in AST, ALT and ALP with control group compared with T1 and T2 and these results agree with Sharifudin *et al.*, [51] and Ouedraogo *et al.* [52] It is likely indicated that *Moringa Oleifera* leaves have good effect on the health status of the rabbits. The all values

of kidney activities were within the normal ranges established [53].

Values of triglyceride were higher significantly ($P < 0.05$) for rabbits in control group than those in T1 and T2. Also, results showed that total cholesterol decreased significantly ($P < 0.05$) by the addition of DMOL in group 3 (19.03%) and group 2 (10.27%) compared to control group, as confirmed by Pari and Kumar, [54] who reported that *Moringa leaves* showed hypocholesterolemic activity. These results of cholesterol values obtained in this study were within the normal physiological range for rabbits (35.0 - 66.0 mg/dl) which reported by [55].

Effects of different levels of DMOL on some haematological parameters of growing New Zealand rabbit serum are presented in **Table 5**. The values of biochemical parameters obtained in this study were in the normal range of values defined for these parameters by previous studies [56] [57] [58] in clinical healthy rabbits. When haematological parameters of control group and group 2 and 3 were compared, white blood cell count, red blood cells and PLT values of control group were significantly lower ($P < 0.05$) than that of T1 and T2. This same trend of result was observed in haemoglobin (Hb) concentration. Similarly, Osman *et al.* [59] indicated that supplementation of rat's feeds with *Moringa oleifera* increased significantly platelets count, RBCs count and hemoglobin (Hb) whereas, RBCs and platelets count increased similarly in rabbits at dose of 300 mg/kg.

This increase in white blood cell count, red blood cells and PLT values and hemoglobin concentration of rabbits may indicate that DMOL is rich in amino acids, vitamins and minerals particularly iron [35] and contain strong antioxidants such as vitamin C [60].

3.2. Caecum Activity and Microbial Activity

The caecum activity and microbial activity as affected by feeding different levels of DMOL of rabbits are presented in **Table 6**. The data from these studies showed that caecal weight and caecal length values of all the treatments were identical to the control ($P > 0.05$). Also, TVFA and caecal pH were significantly ($p > 0.05$) different among treatments. On the contrary, significantly ($P < 0.05$) lowest values of NH3-N (mg/dl cecal juice) were observed in rabbits fed diet 3 (23.84 mg/dl), diet 2 (26.65 mg/dl) and lastly control diet (28.51 mg/dl). The data from these studies showed that the phytogenic compounds of DMOL have shown some positive effects such as regulator of the gut flora.

The total count of bacteria of ceacal content of rabbits in group T2 was significantly ($P < 0.05$) lower than those in control group T0 but similar ($P > 0.05$) to the values of group T1. Therefore, the antimicrobial activity of DMOL may be attributed to the presence of these bioactive compounds which may act on microbiota by inhibiting the growth of microbes, interrupting some metabolic processes, interfering with signal transduction modulation, transcriptional and translational disturbances [61].

Table 6. Caecum activity and microbial activity of growing New Zealand rabbits as affected by feeding dried *Moringa olefira* leaves.

Items	Treatments			± SE
	T0	T1	T2	
Caecal weight, g	169.77a	169.43a	169.73a	0.26
Caecal length, cm	11.98a	11.66a	11.98a	0.18
TVFA (meq./dl cecal juice)	6.12a	6.21a	6.25a	0.07
NH3-N (mg/dl cecal juice)	28.51a	26.65b	23.84c	0.12
Caecal pH	5.97a	5.95a	5.92a	0.05
Bacterial total count (total count × 105)	25.43a	23.72b	22.62b	0.35

a, b, c Means in the same row with different superscripts are significantly different ($P < 0.05$).

Table 7. Carcass characteristics of rabbit groups fed the experimental diets.

Parameters	Treatments			±SE
	T0	T1	T2	
Pre-slaughter weight (g)	2045.93c	2179.68b	2333.42a	9.63
Carcass (%)	46.71c	48.43b	50.02a	0.27
Liver (%)	3.14b	3.45a	3.51a	0.09
Heart (%)	0.31	0.32	0.33	0.01
Kidney (%)	0.59	0.60	0.61	0.03
Total edible parts (TEP), (%)	50.75c	52.80b	54.47a	0.08

a, b and c: Means in the same row having different superscripts differ significantly ($P < 0.05$). Edible giblets (%) = {liver (g) + kidney (g) + heart (g)/pre-slaughter weight (g)}*100, Total edible parts (%) = {carcass weight (g) + weight of edible giblets (g)/pre-slaughter weight (g)}*100.

3.3. Carcass Evaluation of Rabbits

The results of carcass evaluation of rabbits fed experimental diets are presented in Table 7. The present results indicated that there was a significant ($P < 0.05$) effect of dried *Moringa olefira* leaves supplementation on the carcass (%), liver (%) and total edible parts (%) of rabbits across treatments, whereas, treatments T2 and T1 recorded better results than T0. These results may reflect the positively affect the metabolism and immunity of rabbits on the *Moringa olefira*-leaves diets. This observation agrees with Ologhobo *et al.*, [62] where they reported that higher mean values of slaughter weights were recorded for birds fed diets containing *Moringa oleifera* leaf. On the other hand, Dougnon *et al.*, [63] reported that *Moringa olefira* supplementation had no significant effect on the carcass yield.

However, the percent of heart and kidney differences did not show any significance ($P > 0.05$) at the end of the 8 th weeks, however, it was increased marginally with as the inclusion level of *Moringa olefira* leaves increased.

4. Conclusion

Under the condition of the present study, the results suggest that dried *Moringa olefira* leaves supplementation up to 1000 g/Kg diet might improve performance,

bacterial community, antioxidant, biochemical parameters and blood constituents of rabbits.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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